

Altered endothelial reparation and diabetes-Induced endothelial progenitor cell dysfunction

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Abstract

Diabetes mellitus (DM) is considered a leading cause of premature cardiovascular (CV) mortality and morbidity in general population and in individuals with known CV disease. Recent animal and clinical studies have shown that reduced number and weak function of endothelial progenitor cells (EPCs) may not only indicate to higher CV risk, but contribute to the impaired heart and vessels reparation in patients with DM. Moreover, EPCs having a protective impact on the vasculature may mediate the functioning of other organs and systems. Therefore, EPCs dysfunction is probably promising target for DM treatment strategy, while the role of restoring of EPCs number and functionality in CV risk diminish and reduce of DM-related complications is not fully clear. The aim of the review is summary of knowledge regarding EPCs dysfunction in DM patients.

Abbreviations: AGE: advanced glycation end products, BM-EPCs: bone marrow-derived endothelial progenitor cells, CAD: coronary artery disease, CV: cardiovascular, DM: diabetes mellitus, EPCs: endothelial progenitor cells, G-CSF: granulocyte colony-stimulating factor, HIF-1: hypoxia-inducible factor-1, HP1 α : heterochromatin protein 1 α , HUVEC: human umbilical vein endothelial cells, MMPs: metalloproteinases, NADPH: nicotinamide-adenine dinucleotide phosphate, NF-kappaB: nuclear factor kappaB, NO: nitric oxide, PB-EPCs: peripheral blood-derived endothelial progenitor cells, PKC: activation of protein kinase C, RAAS: the renin-angiotensin-aldosterone system, ROS: reactive oxidative species, SDF-1: chemokine stromal cell-derived factor-1, T2DM: type 2 diabetes mellitus, VEGF: vascular endothelial growth factor

Introduction

Diabetes mellitus (DM) is an established risk factor of atherosclerosis, cardiovascular (CV) disease and events, chronic renal disease and heart failure [1-5]. There are several biochemical factors, hemodynamic and endocrine mechanisms with a preponderant initial role in DM-related vascular dysfunction. It is suggested that hyperglycemia, lipotoxicity and hypoxia initiate the microvascular inflammation, induce endothelial dysfunction and worse endothelium [6]. Worsening of intracellular signaling, activation of alternate polyol pathways, increment of growth factors, and accumulation of advanced glycation end products, activation of protein kinase C, activation of the renin-angiotensin-aldosterone system (RAAS), inducing of oxidative stress and apoptosis, decreased nitric oxide (NO) bioavailability, and leukostasis were found an independent causes of weakened endothelium repair ability and worsening of endothelial integrity [7-10]. Finally, co-acting endothelial injury, incompetence in vascular reparation mechanisms and existing co-morbidities (*i.e.*, hypertension, obesity, hyperuricemia, dyslipidemia) may lead to endothelial dysfunction, acceleration of atherosclerosis, senescence, thereby they may negatively influence on CV risk and development of CV disease [11-13].

The key role in the endothelial repair, angiogenesis,

neovascularization and attenuation of vasculature function plays endothelial progenitor cells (EPCs) derived from bone marrow and peripheral blood [14]. In contrast to recently proposed local “response to endothelium injury hypothesis” EPCs, which are mobilized or released into systemic circulation in response to specific stimuli, contribute vessel formation and endothelium reparation directly and through involvement of several paracrine mechanisms [15,16]. Low number and weak functionality of EPC were found in subjects with DM 1 and 2 types, as well as in patients with prediabetes including metabolic syndrome, obesity and insulin resistance [17,18]. In this context, EPCs’ dysfunction is considered a marker of CV risk in general population and in subjects with known DM, as well as a cause of DM-related complications [19]. The aim of the review is summary of knowledge regarding endothelial progenitor cell dysfunction in DM patients.

Determination of endothelial progenitor cells

There are some controversies regarding the definition, origin, determination and isolation of EPCs. Indeed, the definition of EPCs is not standardized completely [20]. On head EPCs were defined as cells positively labeled with both hematopoietic stem cells (CD34) and endothelial cell markers predominantly VEGF receptor-2 (VEGFR2) cumulatively [21]. VEGFR2 is kinase insert domain receptor (KDR) and in follow term “KDR-positive precursor” as a one of important determinations of EPCs has been remained to use optionally.

The later an expression of other hematopoietic stem cells (CD133,

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AC133) markers and endothelial markers (*i.e.*, platelet-endothelial cell adhesion molecule known as CD31, VE-cadherin also known as CD144, caveolin-1, von Willebrand factor, and endothelial NO synthase) on the surface of EPCs was determined [22-26]. Interestingly, several subsets of EPCs may exhibit “non-classical” phenotype expressing CD45, Tei2 and Flt-1 markers [27]. However, it has been found that subset of circulating CD34(+) cells expressing VEGFR2 and CD133 was a phenotypically and functionally distinct population of circulating EPCs that may influence on the reparation and angiogenesis [28].

In the pioneer investigation provided by Hur J et al (2004) [23] two types of EPCs labelled as early EPCs and late outgrowth EPCs and received from the same source were identified. They have expressed different level of CD144, Flt-1, KDR (VEGFR2), and CD45 markers. Late outgrowths EPCs produced more nitric oxide, incorporated more into human umbilical vein ECs monolayer, and are able to better form capillary tube than early EPC. However, early EPC secreted more angiogenic cytokines (VEGF and interleukin-8) than late EPC at culture. Therefore, early EPCs intervened in the monolayer of human umbilical vein endothelial cells (HUVEC), but more late EPCs were incorporated to HUVEC [23]. These suggest that two types of EPC might have different roles in neovasculogenesis and neovascularization. Thus, by now to help classify EPCs, they have used their ability to differentiate into circulating angiogenic cells (referred as early EPCs) and forming endothelial colony cells (referred as late outgrowth EPCs) [28].

Recently investigations have shown that other population of EPCs distinguished from BM-EPCs and was found in the circulation and/or in the tissue (so called “tissue residential EPCs”) may express monocyte marker CD14, together with CD34 or VEGFR2 [29,30]. Interestingly, early EPCs are able to express several monocyte markers, *i.e.*, CD14, CD11b, CD11c, on their surface. Contrary, the populations of late outgrowth EPCs were determined as predominantly CD14-negative and they have usually expressed markers of mature endothelial cells [31]. This “non-classical” phenotype of EPCs has associated with vascular protective capacity and may differentiate into mature endothelial cells under influence of microenvironment in the presence of special growth factors (e.g., VEGF, fibroblast growth factor) and paracrine regulators [32,33].

Despite the exact mechanisms by which EPCs provide cardiovascular protection is unknown [20], both populations of EPCs (early and late outgrowth) are probably to be equal in their ability to produce angiopoietic capacities [34]. However, there is evidence regarding early EPCs could be superior than late outgrowth EPCs to secrete angiopoietic cytokines *in vitro* [23]. Overall, the controversies in heterogeneity of EPCs and their functional capabilities remain uncertain yet.

Definition of endothelial progenitor cell dysfunction

Because of EPCs originated from bone marrow cells and peripheral blood cells maintenance endothelial homeostasis and attenuate the process of angiogenesis and neovascularization, a lot of investigators thought the EPCs as a component of endogenous repair system. Moreover, EPCs having a protective impact on the vasculature may mediate the functioning of other organs and systems [19,20,22,29]. In this context, dysfunction of EPCs defined as wear EPCs functionality (*i.e.*, reduced ability to proliferation, differentiation, adhesion, migration, incorporation into tubular structures, and survival) and/or lowering EPCs' count in the circulation might be a critical step in the initiation of any cause-related vasculopathy that links etiological factors, co-morbidities, aging and clinical events [35]. Nevertheless, EPCs

dysfunction may be a useful predictive tool for evaluating the risk of death in general population and among subjects with known CV and metabolic disease including DM.

The molecular mechanisms of progenitor endothelial cell dysfunction in diabetes mellitus

Alteration of structure and function of the EPCs has identified in type 1 and type 2 DM [20]. The mechanisms underlying EPC reduction in diabetes predominantly include weak bone marrow mobilization, decreased proliferation, and shortened survival [36]. It has suggested that in DM glucose toxicity, lipid toxicity and reactive oxidative species (ROS) via enhancing inflammation may regulate proliferation BM-EPCs [6,9,20]. The key mechanism of this response is the activation of matrix metalloproteinase-9 (MMP-9) through intracellular signal systems, *i.e.*, Akt/STAT and nitric oxide dependent signaling [37]. Maturation and mobbing of the BM-EPCs are under control of growth factors, such as chemokine stromal cell-derived factor-1 (SDF-1), VEGF, granulocyte colony-stimulating factor (G-CSF), and alpha-chemokine that binds to G-protein-coupled CXCR4 [38,39]. Lataillade JJ *et al.* (2000) reported that SDF-1 may stimulate the growth of EPCs' colonies, chemotaxis and cell expansion [39]. Nevertheless, SDF-1 is able to improve survival of the EPCs in culture through suppression of their apoptosis [40]. SDF-1 exerts pleiotropic effects regulating chemoattraction and adhesion of EPCs in CXCR4-dependend mechanisms playing an essential role in the trafficking of EPCs in various tissue including heart and vasculature [41]. It is importantly that SDF-1 gene expression in EPCs and endothelial cells is regulated by the transcription factor hypoxia-inducible factor-1 (HIF-1), which is under control of reduced oxygen tension in the tissues [42]. VEGF has exhibited autocrine action in EPCs suppressing apoptosis and protect a survival effect [43]. GM-CSF may accelerate re-endothelialization and reduce microvascular inflammation through mobbing of EPCs [44]. There is evidence that “residence” EPCs originated from myeloid cells could trans-differentiate into endothelial cells in the same manner [34].

DM is characterized reduced expression of angiopoietic factors (SDF-1, VEGF, G-CSF, CXCR4) in heart and vasculature [18]. Moreover, differentiation and mobbing of EPCs after ischemia-reperfusion injury in DM is defective [46]. The initial role in these processes belongs to over-production of ROS, decreased superoxide dismutase activity, and probably SDF-1 genotype polymorphism [18,42,47], whereas epigenetic changes in EPCs are considered an important mechanism, which links hyperglycemia, lipid toxicity and metabolic memory [6,9,48]. Finally, weak functionality of EPCs in type 1 and type 2 DM is resulting mutual related molecular mechanisms affected cellular signal systems, paracrine regulation and epigenetic modification. Therefore, poor differentiation, mobbing and proliferation of BM-EPCs and PB-EPCs lead to decreased circulating pool of primitive cells and worsening reparative capability [40].

Endothelial progenitor cells and CV risk

Reduced number and weak functionality of circulating EPCs have been demonstrated sufficiently correlation with vascular endothelial dysfunction and independently association with both traditional (aging, hypertension, hypercholesterolaemia, smoking, diabetes, C-reactive protein level) and non-traditional (insulin resistance, adipocyte dysfunction) CV risk factors, Framingham risk factor score [49-51], as well as frequency of major CV events, revascularization, hospitalization rate and death from CV causes [52-54]. Most importantly, EPCs isolated from peripheral blood of the patients with known CAD have exhibited

an impaired migratory and weak proliferative response [50], which have confirmed being of “EPC impaired phenotypes” pre-existing in subjects with CV risk factors prior established CV disease [55,56]. However, the circulating level of EPCs in patients with established higher CV risk is very variable and does not fully correlate with number of CV risk factors [57,58], although number of BM-EPCs has closely predicted asymptomatic atherosclerosis [59,60] and CV disease [61]. One found that lowered number of circulating EPCs originated from bone marrow have accompanied with hypercholesterolemia-induced expression of pro-inflammatory molecules by the vessel wall [62]. Additionally, the level of circulating CD34+KDR+ EPCs may help to identify patients at increased CV risk [55].

However, there are controversies regarding number and colony-forming ability of circulating BM-EPCs and PB-EPCs in individuals with established CV disease and metabolic disease, *i.e.*, DM, metabolic syndrome, obesity. The first controversial reflects disproportion between circulating level of EPCs depending stage of CV disease. To update our knowledge, at the early stage of CV disease especially in patients with asymptomatic atherosclerosis, moderated increase of the EPCs’ count in circulation was found [63]. In contrast, lowered level of EPCs might clarify a severity of atherosclerosis or DM-related vasculopathy [64,65]. The next controversial relates to the level and functionality of EPCs in subjects with obese, metabolic syndrome and DM. Interestingly, at the early stage of metabolically inactive obesity (“not fully” metabolic syndrome) BM-EPCs number may increase and an ability of primitive cells to mobbing, differentiation and colony forming might be not distinguished healthy individuals [66]. In contrast, development of insulin resistance, metabolic syndrome and type 2 DM has closely associated with weak ability of EPCs to mobbing, and the lowered level of circulating EPCs was determined [67]. In contrast, Asnagli V *et al.* (2006) [68] have reported that clonogenic potential of circulating EPCs in patients with type 1 DM may increase. Finally, heterogeneity of EPCs and the variable changes in the EPCs’ phenotypes at the different stages of CV disease and development of DM are limiting factors to determine the predictive value of count and functionality of EPCs in CV risk calculation.

Epigenetic mechanisms of EPCs dysfunction

There is emerging evidence that deregulation of epigenetic features of both BM-EPCs and PB-EPCs could play a key role in DM development and progression [6,9,69]. DNA methylation/hydroxymethylation, histone modifications, and differential expression of specific non-coding RNAs like microRNA (miRNAs) are discussed as causative mechanisms of epigenetic modifications [70]. Despite this, the early phases of epigenetically changes in EPCs are pre-diabetic situation affected mitochondrial injury upon hyperglycemic insult, oxidative stress activation, and lowering survival ability of cell organelles [71]. Indeed, lowered cell membrane protection against hyperglycemic endothelial damage through weak antioxidant effect and insufficient hexosamine biosynthetic pathway may independently increase oxidative stress, amplify endothelial inflammation, and impair endothelial and angiopoietic functions [72]. In this context, even transient hyperglycemia may induce long-lasting activating epigenetic changes in the promoter of the nuclear factor kappaB (NF-kappaB) subunit p65 in EPCs [73]. Moreover, it has found that both the epigenetic changes and the gene expression changes have been persisted for euglycemia and associated with NF-kappaB-induced increasing in monocyte chemoattractant protein 1 and vascular cell adhesion molecule 1 expression, and reducing mitochondrial superoxide production [73]. Interestingly, the up-regulated NFkappaB-p65 gene

might be a determined prior hyperglycemia due to increased histone 3 lysine 4 di- and trimethylation-1 (H3K4m1) and histone demethylase LSD1 but not H3K4m2 or H3K4m3 [74]. Authors concluded that active transcriptional state of the NFkappaB-p65 gene is linked with persisting epigenetic marks, *i.e.*, enhanced H3K4 and reduced H3K9 methylation, which appear to occur as a result of effects of the methyl-writing and methyl-erasing histone enzymes [74]. All these changes have negatively influenced on survival of EPCs inducing their apoptosis, as well as worse of progenitor angiopoietic capability and endothelium integrity [75]. On this way, the dysregulation of epigenetic histone modifications in EPCs associated with increased H3K4m3 and reduced H3K9me3 may accompany to metabolic memory and pro-inflammatory phenotype of resident cells via up-regulation of appropriate miRNAs such as miR-125b, mi34a [76,77]. Indeed, miR-125b mimics increased expression of inflammatory genes, monocyte chemoattractant protein-1, and interleukin-6, and reduced H3K9me3 at their promoters in target cells [76]. miRNA34a overexpression led to a significantly increased EPC senescence, paralleled with an approximately 40% Sirt1 reduction [77]. Yet, dysregulation in H3K4m3 and H3K9me3 has been reported as a cause of increased eNOS expression, which promoters recruitment and differentiation of early EPCs [78].

Probably, pre-existing higher susceptibility of target cells to hyperglycemia-induced oxidative stress in DM might be related to DNA methylation and/or hypomethylation that regulate angiogenic genes through heterochromatin expression [79]. Maeng Y *et al.* (2015) [80] reported that overexpressed heterochromatin protein 1a (HP1a) HP1a in EPCs has promoted the differentiation and angiogenic activity of one *in vitro* and *in vivo*. This effect is mediated by increased expression of angiogenic genes (NOTCH1, cadherin-5, sirtuins, and angiopoietin-like-2), and decreased expression of progenitor cell marker genes (CD133, CXCR4, and C-KIT) [80-82]. Although DNA and histone methylation lead to distinguished repression of heterochromatin (stable long-term repression and local formation, respectively) [83], the crosstalk between SET domain histone methyltransferases and DNA methyltransferases is essential for relationship of both epigenetic pathways, which mediate reprogramming of EPCs in DM [84]. Thus, epigenetic changes are considered a causative factors contributing to apoptosis and senescence in proangiogenic cells and worsening of heart/vessels repairment.

The concept of impaired cardiac and vessel repairment in diabetes: Role of EPCs

EPCs are involved in the homeostasis of the heart and vessels, and their exhaustion or dysfunction may accelerate the course of DM-related CV complications. Because uncommitted progenitor cells can differentiate toward the several phenotypes (*i.e.*, endothelial cell phenotype), it is possible that a broader derangement of immature EPCs predisposes to CV complications in DM [85]. Whether weak functionality and/or lower number of EPCs exist prior to both CV risk and established CV/metabolic diseases or they appear resulting in CV risk factors’ influence on epigenetic mechanisms of immature progenitors is not fully clear. However, an ability of BM-EPCs and PB-EPCs to restore of structure and function of cardiac and vessels in DM is sufficiently impaired [86]. There is evidence regarding potent possibilities to improve the EPCs’ capacity using various methods, *i.e.*, aerobic exercise, lipid lowering drugs, ACE inhibitors, calcium channel blockers, antidiabetic drugs including metformin, sitagliptin [87-92]. Several mechanisms are involved in the attenuation of EPCs functionality by mentioned above approaches, *i.e.*, increase of NO

production via enhancing phosphorylated-AMP-activated protein kinase and phosphorylated-eNOS, down-regulation of high mobility group box-1 and AKT/STAT signaling leading to oxidative stress suppression, attenuation of DNA/histone methylation, inhibition of progenitor cells' apoptosis and NETosis through suppression of proprotein convertase subtilisin/kexin type 9 and Dll4/Notch signaling pathway [93-95]. Subsequently, improving function of EPCs in DM appears to be under control and the prevention of CV complication development could be associated with restoring of EPCs-dependent repair mechanisms [96].

Thus, the EPCs may enhance the balance between heart and vascular injury and repair that is critical for the maintenance of cardiac remodeling, heart function, intimal integrity, endothelial function, and prevention of CV complications [97,98]. All these findings elucidate that EPCs dysfunction could be an alternative therapeutic target to promoting heart and vessels repair in patients with DM. Despite these promising results received in animal and clinical studies, there is not clear whether the EPCs functionality could be restore completely and the circulating number of EPCs would be achieved the level of healthy individuals. By now, the results of the recently performed investigations have shown that EPCs dysfunction in DM remains to be unresolved yet. Probably, there is required more investigations to explain whether EPCs-dependent mechanisms of tissue repair could be exhibited clinically significant relevance or they are an attribute of nonspecific influence of recently known therapeutic approaches.

In conclusion, reduced number and weak function of EPCs may not only indicate to higher CV risk, but contribute to the impaired heart and vessels reparation in patients with DM. EPCs dysfunction is probably promising target for DM treatment strategy, while the role of restoring of EPCs number and functionality in CV risk diminish and reduce of DM-related complications requires more investigations.

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Conflict of interest

None declared

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